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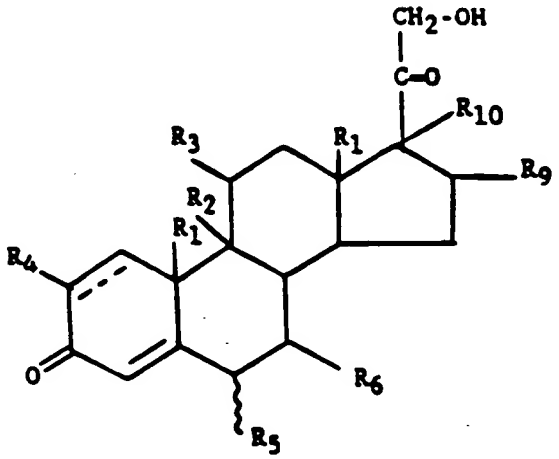

(I)

(57) Abstract

A method of inhibiting hair growth in mammals which comprises the topical administration of a composition comprising an anti-angiogenic effective amount of an angiostatic compound of formula (I). The formula (I) compound is present in an amount of at least 1 mg/ml, preferably from about 1 mg/ml to about 10 mg/ml.

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TOPICAL ANTI-ANGIOGENIC AS HAIR GROWTH INHIBITORS

BACKGROUND OF THE INVENTION

The present invention is a method for inhibiting hair growth through the use of angiostatic steroids. The angiostatic steroid is used in an anti-angiogenesis effective amount.

Angiogenesis is the development of blood vessels which typically would lead to a vascular bed capable of sustaining viable tissue. Angiogenesis is a necessary process in the establishment of embryonic tissue and development of a viable embryo. Similarly angiogenesis is a necessary step in the establishment and development of tumor tissue as well as certain inflammatory conditions. The inhibition of angiogenesis is therefore useful in the control of embryogenesis, inflammatory conditions, and tumor growth, as well as numerous other conditions.

The present invention demonstrates that conversion of a hair follicle from its resting (telogen) into its active (anagen) state can be inhibited by the topical application of angiostatic steroids. One possible explanation is that the conversion of a follicle from the telogen to anagen state required for hair growth is not initiated or possibly that hair growth is stopped in active hair follicles when capillary neogenesis is inhibited.

Information Disclosure Statement

U.S. Patent 4,771,042 describes a novel class of solution stable non-glucocorticoid steroids (angiostatics) which are useful in the inhibition of angiogenesis.

European application No. 83870132.4 (Publication No. 0 114 589) published August 1, 1984, describes the use of cortisone, hydrocortisone and 11α-hydrocortisone in combination with heparin in the inhibition of angiogenesis.

J. Folkman, et al., Science 221, 719-725 (1983), further describes the angiogenesis inhibitory effects of heparin and heparin fragments in combination with cortisone. Folkman further elaborates on the use of heparin or heparin fragments in combination with hydrocortisone in the Proceedings of AACR 26, 384-385 (March 1985).

SUMMARY OF INVENTION

In one aspect the present invention is a method for preventing hair growth through the use of topical angiostatic steroids. The method of the present invention utilizes angiostatic steroids of

general Formula I (see Formula Chart) wherein the various substitutions have the following meanings:

the dotted line between positions C-1 and C-2 means the presence or absence of a second or double bond;

R_1 is CH_3 or C_2H_5;

R_2 is H and R_3 is in the α-position and is -OH, -O-alkyl(C_1-C_{12}), -O-COalkyl(C_1-C_{12}), -O-COaryl, -O-CON(R)$_2$, or OCOOR$_7$ wherein aryl is phenyl wherein f is 0 to 2 and wherein the phenyl ring is -$(CH_2)_f$- optionally substituted with from 1 to 3 groups selected from Cl, F, Br, alkyl(C_1-C_3), alkoxy(C_1-C_3), thioalkoxy(C_1-C_3), i.e., -S-alkyl-(C_1-C_3), Cl_3C-, F_3C, NH_2, and -$NHCOCH_3$, i.e., acetamido, or aryl is furyl, thienyl, pyrrolyl or pyridyl each of said hetero moiety being optionally substituted with one or two C_1-C_4 alkyl groups, and wherein R is hydrogen, alkyl(C_1-C_4), or phenyl and each R can be the same or different; wherein R_7 is aryl as defined herein or alkyl(C_1-C_{12}); or

R_2 is α-Cl and R_3 is β-Cl; or

R_2 and R_3 taken together form an oxygen (-O-) bridging positions C-9 and C-11; or

R_2 and R_3 taken together form a second or a double bond between positions C-9 and C-11;

R_4 is H, CH_3, Cl or F;

R_5 is H, OH, F, Cl, Br, CH_3, phenyl, vinyl or allyl;

R_6 is H or CH_3;

R_9 is H, OH, CH_3, F or $=CH_2$; and

R_{10} is H, OH, CH_3 or R_{10} forms a second or double bond between positions C-16 and C-17.

The present invention provides a method of inhibiting hair growth in mammals and more preferably in a humans which comprises the topical administration of a composition comprising an angiostatic steroid of Formula I. The compound of Formula I is present in an anti-angiostatic effective amount, preferably at least 1 mg/ml or from about 1 mg/ml to about 10 mg/ml.

DETAILED DESCRIPTION OF INVENTION

The present invention is directed toward a method for preventing hair growth by the topical administration of an effective amount of an angiostatic steroid. The angiostatic steroid is present in an anti-angiogenic "effective amount" which is an amount effective to

prevent the development of capillary blood vessels in the hair follicle. This method provides an easy means to prevent hair growth in a hair follicle of a mammal including humans. The method is effective as long as the topical administration is continued and therefore discontinued use will allow normal hair regrowth.

There are both medical and cosmetic indications for the subject method. Medical indications include hereditary and acquired hypertrichotic disorders such as hypertrichosis lanuginosa, virilizing and nonvirilizing hirsutism and postmenopausal hypertrichosis. Cosmetic indications would appear to be almost limitless, restricted only by safety and efficacy considerations.

Preferred embodiments of the present invention are the topical administration of an angiostatic steroid as represented by Formulas I to VI wherein the groups have the following meanings:

In Formula I a preferred structure is where R_1 through R_4 and R_6 are hydrogen, R_5 is fluorine, R_9 is methyl and R_{10} is hydroxyl. In Formulas II to VI each of R_4, R_7 and R_8 is hydrogen; R_1 is alkyl(C_1-C_3), preferably CH_3 or C_2H_5; R_5 is CH_3, F, Cl, Br, H or OH, and more preferably R_5 is in the α-position and is CH_3, H or F; R_6 is H or CH_3 and more preferably is H; R_9 is H, α-OH or CH_3; and R_{10} is α-H or α-OH. Additionally in Formula III R_2 is hydrogen and R_3 is in the α-position and is OH, -O-alkyl(C_1-C_{12}), preferably -O-alkyl(C_1-C_4), -O-COalkyl(C_1-C_{12}), preferably -O-COalkyl(C_1-C_6), -O-COaryl, -O-CON(R)$_2$ or -OCOOR$_7$ wherein aryl, R, and R_7 have the meanings defined in Formula I and preferably aryl is phenyl and R is hydrogen or methyl. In Formula I the wavy bond means the group represented by R_5 may be in the alpha or beta position.

The aryl moiety in the R_3 group -OCOaryl is attached to the carbonyloxy moiety through any of the available ring carbon atoms of said aryl moiety.

Any reference herein to compounds of Formula I includes pharmacologically acceptable salts thereof.

In practicing the present invention the steroids of Formula I-VI are topically administered to an area on the body where hair growth is to be inhibited. The topical administration of the angiostatic steroid composition is typically done by routine applications to the hair follicles where hair growth is undesirable. The angiostatic steroid is generally present in an amount effective to have an anti-

angiostatic result, preferably at least 1 mg/ml or from about 1 mg/ml to about 10 mg/ml. The dosage is, of course, dependent upon the circumstance of treatment and particular mammal treated which can be readily determined.

Sterile aqueous solutions of the compounds of Formula I typically will contain other components such as preservatives, anti-oxidants, chelating agents, or other stabilizers. Suitable preservatives can include benzyl alcohol, the parabens, benzalkonium chloride, or benzoic acid. Anti-oxidants such as sodium bisulfite, ascorbic acid, propyl 3,4,5-trihydroxy benzoate, and the like may be employed. Chelating agents such as citrate, tartrate, or ethylenediaminetetraacetic acid (EDTA) may be used. Other additives useful as stabilizers of corticosteroid prodrugs (e.g., creatinine, polysorbate 80, and the like) may be employed.

Sterile aqueous solutions of compounds of Formula I can be administered to the patent being treated, or various topical formulations can be prepared.

Typical topical compositions include those pharmaceutical forms in which can be applied externally by direct contact with the surface to be treated. Conventional pharmaceutical forms for this purpose include ointments, waxes, lotions, pastes, jellies, sprays, aerosols, and the like in aqueous or nonaqueous formulations. The term "ointment" embraces formulations (including creams) having oleaginous, absorption, water-soluble and emulsion-type bases, e.g., petrolatum, lanolin, polyethylene glycols, as well as mixtures of these.

Preparation of topical compositions are disclosed in U.S. Patents 4,139,619 and 4,596,812, both herein incorporated by reference.

The composition can be applied to the area to be treated, such as the scalp, face, arms, legs or other areas where hair growth is undesirable, by spraying, dabbing or swabbing. Other less specific methods can be employed provided the active ingredient, angiostatic steroid is delivered to the region of the hair follicle. Preferably, the composition is periodically applied to the treatment area on a routine basis prior to, during and subsequent to the inhibitions of hair growth. Generally, the routine treatment would be to apply the composition at least daily, preferably twice daily. When hair growth

is desired the treatment can be discontinued.

The compounds of Formula I are known in the art or are prepared by procedures known in the art, such as described in U.S. Patent 4,771,042 herein incorporated by reference. Illustrative examples of the compounds of Formula I include: 17α,21-dihydroxy-4,9(11)pregnadiene-3,20-dione; 6α-methylcortisone; 17α,21-dihydroxy-6α-methylpregna-1,4,9(11)-triene-3,20-dione; and 11-epi-cortisol, and 6α-fluoro-17α-hydroxy-21-hydroxy-16β-methylpregna-4,9(11)-diene-3,20-dione.

Example 1

The prevention of hair growth through the application of an angiostatic steroid was tested as follows.

Groups of synchronously cycling, age matched male mice were shaved to evaluate hair growth status and subsequently treated topically with an acetone solution of a compound selected from Formula I. Evaluating the growth of hair in a pigmented mouse young enough to remain in a synchronous hair cycle (less than 4 months old) is relatively easy to score, since pigment in mouse skin is exclusively associated with the anagen bulb and hair shaft and consequently is present in large amounts only in anagen. Thus, when a pigmented mouse is shaved in telogen, the skin is pink and remains so until the onset of the next anagen which is recognized by a gradual darkening of the skin corresponding to the proliferation of melanocytes and the production of melanin in the anagen hair bulb. Inhibition of anagen (hair growth) is recognizable as a pink spot surrounded by pigmented skin.

In this experiment the compound selected from Formula I was 6α-fluoro-17α-hydroxy-21-hydroxy-16β-methylpregna-4,9(11)-diene-3,20-dione (Formula I wherein R_1 through R_4 and R_6 are hydrogen, R_5 is fluorine, R_9 is methyl and R_{10} is hydroxyl). This compound was topically applied to the mouse's shaved back once daily, commencing at 22 days of age and continuing through 35 days of age, an interval corresponding to the first rest and second anagen in this species. Control groups received either the vehicle or nothing. The timing, extent and pattern of hair growth was evaluated subjectively and by sequential photographs. A treatment related inhibition of hair growth was observed that corresponded with the area of drug applica-

tion. The inhibitory effect occurred without clinical evidence of dermatitis, suggesting that inhibition was follicle specific and not due to local or systemic toxicity. The effect was fully reversible, in that with cessation of treatment, follicles slowly entered anagen from skin areas that had been previously without visible hair growth.

These observations indicate that 6α-fluoro-17α-hydroxy-21-hydroxy-16β-methylpregna-4,9(11)-diene-3,20-dione and related compounds,(for example, the enatiomer where R_9 is a β-methyl) in the absence of exogenous co-factors such as heparin and related materials, can inhibit murine hair growth after topical administration, and that the effect is fully reversible and is not associated with overt evidence of irritation or systemic toxicity.

Example 2

A study was conducted as in Example 1, using varying amounts of 6α-fluoro-17α-hydroxy-21-hydroxy-16β-methylpregna-4,9(11)-diene--3,20-dione. The results are shown in Table 1 which scores the mice each day for evidence of hair growth on the treated area. The scores are number of mice in group with regrowth in treatment area per number of mice in treatment group.

TABLE 1

	Study Day							
Treatment	10	11	12	13	14	15	16	17
None	0/5	3/5	4/5	4/5	4/5	4/5	5/5	5/5
Vehicle	0/5	1/5	1/5	2/5	3/5	3/5	3/5	3/5
1 mg/ml	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
2 mg/ml	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
4 mg.ml	0/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5

Clear evidence of inhibition was observed in treated groups at all dosages versus the control ("none") and "vehicle."

Histologic examination of the transition area between the treated site and the adjacent skin revealed that follicles were arrested either in telogen or early anagen in areas without visible evidence of hair growth. Of some interest was the absence of mature fat cells in treated skin. Scattered small adipocytes were present

but were greatly reduced relative to the adjacent untreated skin. The vasculature was not affected in treated skin. Those vessels that were perfused before treatment began remain intact which is consistent with the theory that the mechanism of activity is specific for newly forming capillaries. Evidence of injury to existing capillaries, such as hemorrhage, edema, endothelial swelling or perivascular inflammation was not observed. Neither clinical nor histologic evidence of skin irritation was observed.

Example 3

Since the study in Example 2 showed hair inhibition at all levels a similar study was conducted to determine a minimum dosage. The same compound was evaluated and the results are shown in Table 2.

TABLE 2

	Observation Day												
Treatment	2	3	4	5	6	7	8	9	10	11	12	13	14
None	0/5	1/5	1/5	3/5	4/5	4/5	4/5	4/5	5/5	5/5	5/5	5/5	5/5
Vehicle	0/5	1/5	1/5	4/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
0.01 mg/ml	0/5	1/5	1/5	3/5	4/5	4/5	4/5	4/5	5/5	5/5	5/5	5/5	5/5
0.1 mg/ml	0/5	0/5	0/5	3/5	4/5	4/5	4/5	4/5	4/5	5/5	5/5	5/5	5/5
1.0 mg/ml	0/4	0/4	0/4	0/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4	2/4	4/4

Evidence of hair growth inhibition was seen at 1.0 mg/ml but not at 0.1 mg/ml or lower concentrations.

Example 4

The same hair growth study as used in Example 1 was employed to compare three steroid compounds. Two compounds were selected from Formula I (A: 6α-fluoro-17α-hydroxy-21-hydroxy-16β-methylpregna-4,9(11)-diene-3,20-dione, B: 6α-fluoro-17α-hydroxy-21-hydroxy--16α-methylpregna-4,9(11)-diene-3,20-dione, and the third compound was a metabolite C: 6α-fluoro-17α-hydroxy-16-methyl-3,20-dioxo-pregna-4,9(11)-dien-21-oic acid).

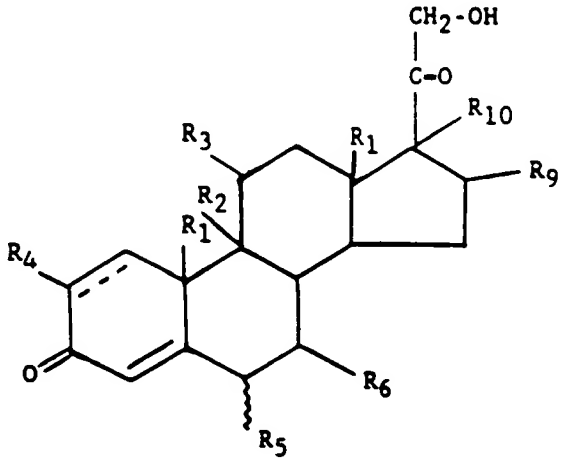
These compounds were tested as for inhibitory activity at 1 mg/ml concentrations in acetone, and the results are summarized in Table 3 below.

TABLE 3

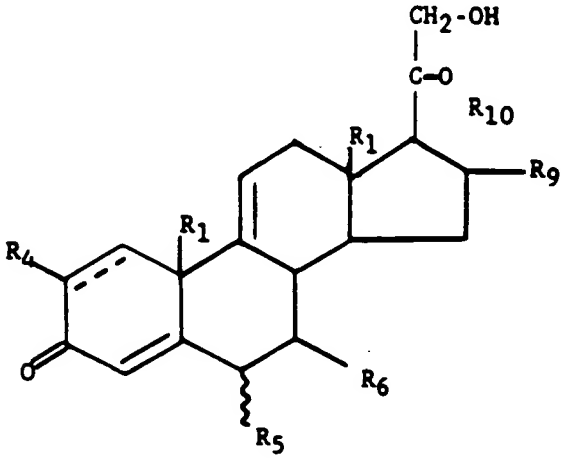
Treatment	Study Day 10	11	12	13	14	15
None	0/5	2/5	3/5	5/5	5/5	5/5
Vehicle	0/4	3/4	3/4	3/4	4/4	4/4
A	0/5	0/5	0/5	0/5	0/5	1/5
B	0/5	0/5	0/5	0/5	0/5	0/5
C	0/5	0/5	3/5	5/5	5/5	5/5

Again, clear evidence of inhibition was observed in A and B (formulas of the subject invention) treated groups. No inhibitory activity was observed for the metabolite C (not a formula of the subject invention).

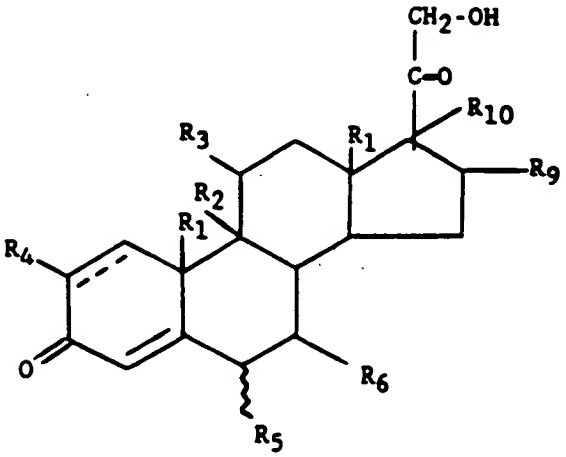
FORMULA CHART



FORMULA I

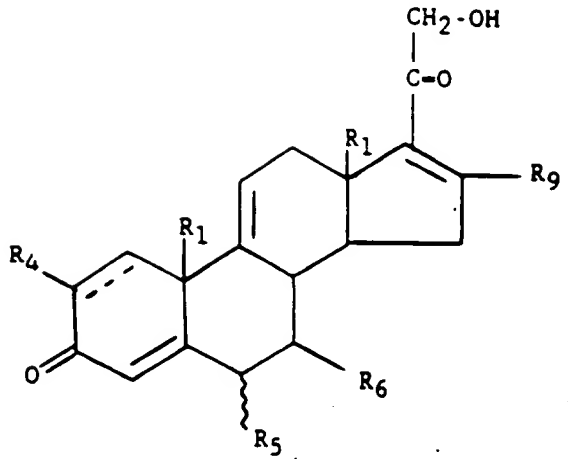


FORMULA II

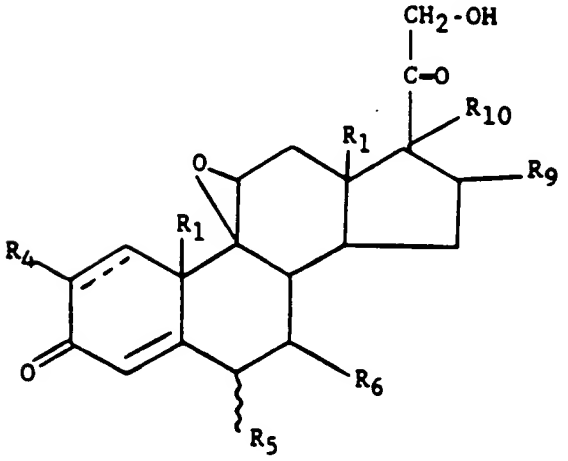


FORMULA III

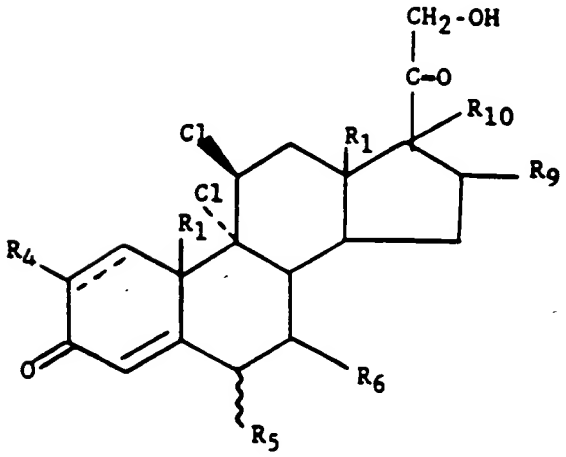
FORMULA CHART (continued)



FORMULA IV



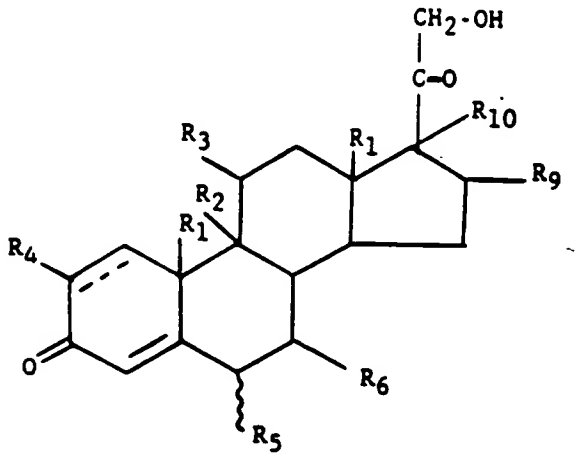
FORMULA V



FORMULA VI

CLAIMS

1. A use of a compound of Formula I for the manufacture of a medicament for inhibiting hair growth in mammals comprising the topical administration of a composition comprising an anti-angiogenic effective amount of a compound of Formula I:



FORMULA I

and pharmacologically acceptable salts thereof, wherein the dotted line between positions C-1 and C-2 means the presence or absence of a double bond; the bond at C-6 denotes α or β;

wherein R_1 is CH_3 or $-C_2H_5$;

wherein R_2 is H, and R_3 is in the α-position and is -OH, -O-alkyl-(C_1-C_{12}), $-OC(=O)alkyl(C_1\text{-}C_{12})$, $-OC(=O)aryl$, $-OC(=O)N(R)_2$, or $-OC(=O)OR_7$,

wherein aryl is furyl, thienyl, pyrrolyl, or pyridyl wherein each of said hetero moiety is optionally substituted with one or two (C_1-C_4)-alkyl groups or aryl is $-(CH_2)_f$-phenyl wherein f is 0 to 2 and wherein the phenyl ring is optionally substituted with one to three groups selected from chlorine, fluorine, bromine, $alkyl(C_1\text{-}C_3)$, $alkoxy(C_1\text{-}C_3)$, $thioalkoxy(C_1\text{-}C_3)$, Cl_3C-, F_3C-, $-NH_2$ and $-NHCOCH_3$ and wherein R is hydrogen, $alkyl(C_1\text{-}C_4)$, or phenyl and each R can be the same or different; and wherein R_7 is aryl as herein defined or $alkyl(C_1\text{-}C_{12})$; or

wherein R_2 is α-Cl and R_3 is β-Cl; or

wherein R_2 and R_3 taken together are oxygen (-O-) bridging positions C-9 and C-11; or

wherein R_2 and R_3 taken together form a double bond between positions C-9 and C-11;

wherein R_4 is H, CH_3, Cl or F;
wherein R_5 is H, OH, F, Cl, Br, CH_3, phenyl, vinyl or allyl;
wherein R_6 is H or Cn_3;
wherein R_9 is H, OH, CH_3, F or $-CH_2$; and
wherein R_{10} is H, OH, CH_3 or R_{10} forms a second bond between positions C-16 and C-17.

2. The use of claim 1 wherein R_1, R_2, R_3, R_4, and R_6 are hydrogen, R_5 is fluorine, R_9 is methyl and R_{10} is hydroxyl.

3. The use of claim 2 wherein R_9 is α-methyl.

4. The use of claim 2 wherein R_9 is β-methyl.

5. The use of Claim 1 wherein said composition comprises at least 1 mg/ml of a compound of Formula I.

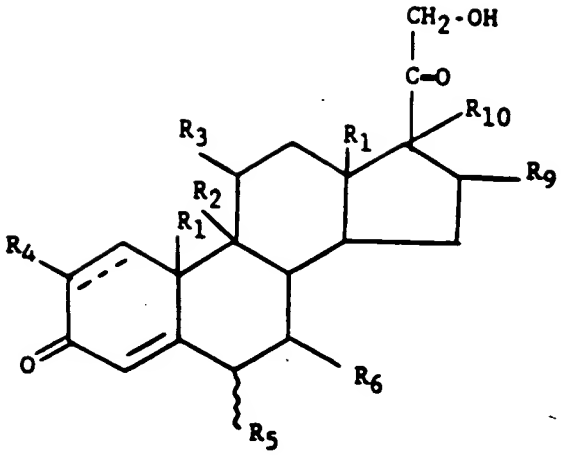
6. The use of Claim 5 wherein said compound of Formula I is present in an amount of from about 1 to about 10 mg/ml.

7. The use of Claim 1 which includes in said composition a pharmaceutical carrier adapted for topical application.

8. The use of Claim 1 wherein said composition is routinely applied to an area of treatment.

9. The use of Claim 8 wherein said composition is applied daily or twice daily.

10. A pharmaceutical composition comprising:
an anti-angiogenic effective amount of a compound of Formula I:



FORMULA I

and pharmacologically acceptable salts thereof, wherein the dotted line between positions C-1 and C-2 means the presence or absence of a double bond; the bond at C-6 denotes α or β;

wherein R_1 is CH_3 or $-C_2H_5$;

wherein R_2 is H, and R_3 is in the α-position and is -OH, -O-alkyl-(C_1-C_{12}), $-OC(=O)alkyl(C_1-C_{12})$, $-OC(=O)aryl$, $-OC(=O)N(R)_2$, or $-OC(=O)OR_7$,

wherein aryl is furyl, thienyl, pyrrolyl, or pyridyl wherein each of said hetero moiety is optionally substituted with one or two (C_1-C_4)-alkyl groups or aryl is $-(CH_2)_f$-phenyl wherein f is 0 to 2 and wherein the phenyl ring is optionally substituted with one to three groups selected from chlorine, fluorine, bromine, $alkyl(C_1-C_3)$, $alkoxy(C_1-C_3)$, $thioalkoxy(C_1-C_3)$, Cl_3C-, F_3C-, $-NH_2$ and $-NHCOCH_3$ and wherein R is hydrogen, $alkyl(C_1-C_4)$, or phenyl and each R can be the same or different; and wherein R_7 is aryl as herein defined or $alkyl(C_1-C_{12})$; or

wherein R_2 is α-Cl and R_3 is β-Cl; or

wherein R_2 and R_3 taken together are oxygen (-O-) bridging positions C-9 and C-11; or

wherein R_2 and R_3 taken together form a double bond between positions C-9 and C-11;

wherein R_4 is H, CH_3, Cl or F;

wherein R_5 is H, OH, F, Cl, Br, CH_3, phenyl, vinyl or allyl;

wherein R_6 is H or CH_3;

wherein R_9 is H, OH, CH_3, F or $=CH_2$; and

wherein R_{10} is H, OH, CH_3 or R_{10} forms a second bond between positions C-16 and C-17; and

a pharmaceutical carrier adapted for topical application.

11. The composition of Claim 10 wherein R_1, R_2, R_3, R_4, and R_6 are hydrogen, R_5 is fluorine, R_9 is methyl and R_{10} is hydroxyl.

12. The composition of Claim 11 wherein R_9 is α-methyl.

13. The composition of Claim 11 wherein R_9 is β-methyl.

14. The composition of Claim 10 wherein said composition comprises at least 1 mg/ml of a compound of Formula I.

15. The composition of Claim 14 wherein said compound of Formula I is present in an amount of from about 1 to about 10 mg/ml.